09/246,457

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(FILE 'HOME' ENTERED AT 09:50:48 ON 23 MAY 2001)

INDEX 'ADISALERTS, ADISINSIGHT, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, DRUGNL, ...' ENTERED AT 09:51:19 ON 23 MAY 2001

SEA CYTOCHROME P450

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     FILE 'MEDLINE, EMBASE, CAPLUS, SCISEARCH, BIOSIS' ENTERED AT 09:53:33 ON
     23 MAY 2001
L2           1469 S L1 AND VARIANT
L3              0 S L2 AND (POSITION 331 OR POSITION 280 OR POSITION 242)
L4             14 S L2 AND (331 OR 280 OR 242)
L5              6 DUP REM L4 (8 DUPLICATES REMOVED)
L6            552 S L1 AND P450CAM
L7             52 S L6 AND VARIA?
L8              0 S L7 AND (331 OR 280 OR 242)
L9             26 S L7 AND (STAB? OR CATALYT?)
L10             8 DUP REM L9 (18 DUPLICATES REMOVED)
L11            18 DUP REM L7 (34 DUPLICATES REMOVED)
L12             4 S L2 AND NAPHTHALENE
L13             1 DUP REM L12 (3 DUPLICATES REMOVED)
L14             4 S L2 AND (HORSERADISH(W)PEROXIDASE)
L15             1 DUP REM L14 (3 DUPLICATES REMOVED)
L16            67 S L2 AND (NADH OR DELTA(W)AMINOLEVU? OR ALA)
L17            29 DUP REM L16 (38 DUPLICATES REMOVED)
L18             0 S L17 AND (331 OR 280 OR 242)
L19           131 S L2 AND AROMAT?
L20             6 S L19 AND (CAMPHOR OR NAPHTHALENE)
L21             2 DUP REM L20 (4 DUPLICATES REMOVED)
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=> d l5 ibib ab 1-6

L5 ANSWER 1 OF 6 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999139982 EMBASE
TITLE: A refined model for the solution structure of oxidized putidaredoxin.
AUTHOR: Pochapsky T.C.; Jain N.U.; Kuti M.; Lyons T.A.; Heymont J.
CORPORATE SOURCE: T.C. Pochapsky, Department of Chemistry, Brandeis University, Waltham, MA 02254-9110, United States. pochapsky@binah.cc.brandeis.edu
SOURCE: Biochemistry, (13 Apr 1999) 38/15 (4681-4690).
Refs: 54
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A refined model for the solution structure of oxidized putidaredoxin (Pdx(o)), a Cys4Fe2S2 ferredoxin, has been determined. A previous structure (Pochapsky et al. (1994) Biochemistry 33, 6424-6432; PDB entry 1PUT) was calculated using the results of homonuclear two-dimensional NMR experiments. New data has made it possible to calculate a refinement of the original Pdx(o) solution structure. First, essentially complete assignments for diamagnetic 15N and 13C resonances of Pdx(o) have been made using multidimensional NMR methods, and 15N- and 13C-resolved NOESY experiments have permitted the identification of many new NOE restraints for structural calculations. Stereospecific assignments for leucine and valine CH3 resonances were made using biosynthetically directed fractional 13C labeling, improving the precision of NOE restraints involving these residues. Backbone dihedral angle restraints have been obtained using a combination of two-dimensional J-modulated 15N, 1H HSQC and 3D (HN)-CO(CO)NH experiments. Second, the solution structure of a diamagnetic form of Pdx, that of the C85S **variant** of gallium putidaredoxin, in which a nonligand Cys is replaced by Ser, has been determined (Pochapsky et al. (1998) J. Biomol. NMR 12, 407- 415), providing information concerning structural features not observable in the native ferredoxin due to paramagnetism. Third, a crystal structure of a closely related ferredoxin, bovine adrenodoxin, has been published (Muller et al. (1998) Structure 6, 269-**280**). This structure has been used to model the metal binding site structure in Pdx. A family of fourteen structures is presented that exhibits an rmsd of 0.51 .ANG. for backbone heavy atoms and 0.83 .ANG. for all heavy atoms. Exclusion of the modeled metal binding loop region reduces overall the rmsd to 0.30 for backbone atoms and 0.71 .ANG. for all heavy atoms.

L5 ANSWER 2 OF 6 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 1
ACCESSION NUMBER: 1999344550 EMBASE
TITLE: Evidence for polymorphism in the canine metabolism of the cyclooxygenase 2 inhibitor, celecoxib.
AUTHOR: Paulson S.K.; Engel L.; Reitz B.; Bolten S.; Burton E.G.; Maziasz T.J.; Yan B.; Schoenhard G.L.
CORPORATE SOURCE: Dr. S.K. Paulson, G.D. Searle, 4901 Searle Parkway, Skokie, IL 60077, United States. Susan.K.Paulson@monsanto.com
SOURCE: Drug Metabolism and Disposition, (1999) 27/10 (1133-1142).

Refs: 27
ISSN: 0090-9556 CODEN: DMDSAI
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The pharmacokinetics of celecoxib, a cyclooxygenase-2 inhibitor, was characterized in beagle dogs. Celecoxib is extensively metabolized by dogs to a hydroxymethyl metabolite with subsequent oxidization to the carboxylic acid analog. There are at least two populations of dogs, distinguished by their capacity to eliminate celecoxib from plasma at either a fast or a slow rate after i.v. administration. Within a population of **242** animals, 45.0% were of the EM phenotype, 53.5% were of the PM phenotype, and 1.65% could not be adequately characterized.

The mean (.+-.S.D.) plasma elimination half-life and clearance of celecoxib were 1.72 .+-. 0.79 h and 18.2 .+-. 6.4 ml/min/kg for EM dogs and 5.18 .+-. 1.29 h and 7.15 .+-. 1.41 ml/min/kg for PM dogs. Hepatic microsomes from EM dogs metabolized celecoxib at a higher rate than microsomes from PM dogs. The cDNA for canine cytochrome P-450 (CYP) enzymes, CYP2B11, CYP2C21, CYP2D15, and CYP3A12 were cloned and expressed in sf 9 insect cells. Three new **variants** of CYP2D15 as well as a novel **variant** of CYP3A12 were identified. Canine rCYP2D15 and its **variants**, but not CYP2B11, CPY2C21, and CYP3A12, readily metabolized celecoxib. Quinidine (a specific CYP2D inhibitor) prevented celecoxib metabolism in dog hepatic microsomes, providing evidence of a predominant role for the CYP2D subfamily in canine celecoxib metabolism. However, the lack of a correlation between celecoxib and bufuralol metabolism in hepatic EM or PM microsomes indicates that other CYP subfamilies besides CYP2D may contribute to the polymorphism in canine celecoxib metabolism.

L5 ANSWER 3 OF 6 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999041201 MEDLINE
DOCUMENT NUMBER: 99041201 PubMed ID: 9825828
TITLE: Comparisons between in-vitro and in-vivo metabolism of (S)-warfarin: catalytic activities of cDNA-expressed CYP2C9, its Leu359 **variant** and their mixture versus unbound clearance in patients with the corresponding CYP2C9 genotypes.
AUTHOR: Takahashi H; Kashima T; Nomoto S; Iwade K; Tainaka H; Shimizu T; Nomizo Y; Muramoto N; Kimura S; Echizen H
CORPORATE SOURCE: Department of Pharmacotherapy, Meiji Pharmaceutical University, Kiyare, Tokyo, Japan.
SOURCE: PHARMACOGENETICS, (1998 Oct) 8 (5) 365-73.
Journal code: BRT; 9211735. ISSN: 0960-314X.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990128
Last Updated on STN: 19990128
Entered Medline: 19990114

AB To study whether an in-vitro model for three different genotypes of human CYP2C9*3 polymorphism would be useful for predicting the in-vivo kinetics of (S)-warfarin in patients with the corresponding genotypes, the intrinsic clearance (Cl(int) or Vmax/Km) for (S)-warfarin 7-hydroxylation obtained from recombinant human CYP2C9*1 [wild-type (wt)] and CYP2C9*3 (Leu359/Leu) expressed in yeast and the mixture of equal amounts of these were compared with the in-vivo unbound oral CI (CI(po,u)) of (S)-warfarin obtained from 47 Japanese cardiac patients with the corresponding CYP2C9

genotypes. The in-vitro study revealed that the recombinant CYP2C9*1 (wt/wt), 2C9*3 (L[redacted]59/Leu) and their mixture (Ile[redacted]/Leu) possessed a mean Km of 2.6, 10.4 and 6.6 microM and Vmax of **280**, 67 and 246 pmol/min/nmol P450, respectively. Thus, the mean in-vitro Cl(int) obtained from recombinant CYP2C9*3 (Leu359/Leu) and the mixture (Ile359/Leu) of 2C9*3 and 2C9*1 were 94% and 65% lower than that obtained from CYP2C9*1 (wt/wt) (6.7 versus 38 versus 108 ml/min/micromol P450, respectively). The in-vivo study showed that the median Cl(po,u) for (S)-warfarin obtained from patients with homozygous (Leu359/Leu, n = 1) and heterozygous (Ile359/Leu, n = 4) CYP2C9*3 mutations were reduced by 90% (62 ml/min) and 66% (212 ml/min, $P < 0.05$) compared with that obtained from those with homozygous 2C9*1 (625 ml/min, n = 42). Consequently, there was a significant correlation ($r = 0.99$, $P < 0.05$) between the in-vitro Cl(int) for (S)-warfarin 7-hydroxylation and the in-vivo Cl(po,u) for (S)-warfarin in relation to the CYP2C9*3 polymorphism. In conclusion, the in-vitro model for human CYP2C9*3 polymorphism using recombinant **cytochrome P450** proteins would serve as a useful means for predicting changes in in-vivo kinetics for (S)-warfarin and possibly other CYP2C9 substrates in relation to CYP2C9*3 polymorphism.

L5 ANSWER 4 OF 6 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 3
ACCESSION NUMBER: 95287854 EMBASE
DOCUMENT NUMBER: 1995287854
TITLE: Trans effects on cysteine ligation in the proximal His93Cys **variant** of horse heart myoglobin.
AUTHOR: Hildebrand D.P.; Ferrer J.C.; Tang H.-L.; Smith M.; Mauk A.G.
CORPORATE SOURCE: Dept. of Biochem./Molecular Biology, University of British Columbia,Vancouver, BC V6T 1Z3, Canada
SOURCE: Biochemistry, (1995) 34/36 (11598-11605).
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Three **variants** of horse heart myoglobin (Mb) in which the proximal His93 residue has been replaced with a Cys residue have been constructed and studied by NMR, EPR, and MCD spectroscopy to evaluate the contributions of proximal and distal residues to the coordination environment of the heme iron in these proteins. Although no experimental conditions were identified that allowed quantitative ligation of the cysteine residue to the heme iron in the His93Cys **variant**, all of the spectroscopic evidence collected for the His93Cys/His64Ile and His93Cys/His64Val double **variants** supports the assignment of thiolate as the ligand to iron in the oxidized forms of these **variants**. The double metMb **variants** exhibit Soret maxima that are considerably blue-shifted. 1H NMR spectra with decreased mean methyl resonances, and EPR spectra with highly rhombic g values. These spectroscopic data for the Fe(III) **variants** resemble the corresponding properties reported for ferricytochrome P-450. The decrease in the reduction potential of the double **variants** by **280** mV relative to wild-type protein is also consistent with the low midpoint potential of **cytochrome P450**. MCD spectroscopy of these **variants** confirms that the proximal cysteine residue is not bound in the reduced forms of these proteins and, in the case of the His93Cys **variant**, that the distal histidine is coordinated to the iron. Similar coordination environments were created in the ferrimyoglobin **variants** by cyanogen bromide modification, which resulted in cyanation of the sulfur atom and prevented the ligation of Cys93 to the heme iron. From these results it is apparent that simple

mutagenic modifications of the active site of horse heart myoglobin can reproduce the cha[redacted]teristics of ferricytochrome P[redacted]0, but that reproduction of the spectroscopic properties of ferrocytochrome P-450 will require more subtle modifications.

L5 ANSWER 5 OF 6 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 4
ACCESSION NUMBER: 95252188 EMBASE
DOCUMENT NUMBER: 1995252188
TITLE: Comparison of substrate metabolism by wild type CYP2D6 protein and a **variant** containing methionine, neat valine, at position 374.
AUTHOR: Crespi C.L.; Steimel D.T.; Penman B.W.; Korzekwa K.R.; Fernandez-Salguero P.; Buters J.T.M.; Gelboin H.V.; Gonzalez F.J.; Idle J.R.; Daly A.K.
CORPORATE SOURCE: GENTEST Corporation, 6 Henshaw Street,Woburn, MA 01801, United States
SOURCE: Pharmacogenetics, (1995) 5/4 (234-243).
ISSN: 0960-314X CODEN: PHMCEE
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
052 Toxicology
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We have analysed kinetic parameters of cDNA-derived CYP2D6 proteins derived from the original CYP2D6 cDNA isolate (Gonzalez FJ et al. Nature 1988: **331**, 442-446) which contains methionine at position 374 (CYP2D6-Met) and a modified cDNA which contains valine at position 374 (CYP2D6-Val). This latter protein is predicted from the CYP2D6 genomic sequence. Several quantitative differences, but no qualitative differences in metabolism were observed. CYP2D6-Met was found to have a two-fold lower K(m) and a threefold lower turnover rate for (R)(+)-bufuralol 1'-hydroxylation as compared to CYP2D6-Val. In contrast, CYP2D6-Met and CYP2D6-Val had a similar K(m) for debrisoquine 4-hydroxylation while CYP2D6-Val had an 18-fold higher turnover rate. CYP2D6-Val and CYP2D6-Met had similar K(m)s for metoprolol but CYP2D6-Val showed a three-fold higher capacity for the O-demethylation reaction compared to .alpha.-hydroxylation which is more similar to that seen in human liver. In the case of sparteine, CYP2D6-Val and CYP2D6-Met showed similar capacities for formation of the 2-dehydrosparteine metabolite but the K((i) value for CYP2D6-Met was six-fold higher than that for CYP2D6-Val. Kinetic differences between CYP2D6-Met and CYP2D6-Val were further probed by examination of apparent K(i) for inhibition of (R,S)(+)-bufuralol 1'-hydroxylation. Similar K(i) values (within a factor of three) were observed for perhexiline and (R,S)-propranolol while quinidine and dextromethorphan were 8.5-fold and 21-fold more effective inhibitors of CYP2D6-Val relative to CYP2D6-Met. An allele specific polymerase chain reaction assay was developed for the CYP2D6-Met allele. The CYP2D6-Met allele was not found among 83 individuals. In the aggregate, these data indicated that the CYP2D6-Val allele is the more common allele in human populations. The quantitative kinetic differences between these two enzymes appears most pronounced for substrates/inhibitors with rigid structures. CYP2D6-Val more often has a substantially lower K(m) and/or a substantially higher capacity to metabolize those substrates.

L5 ANSWER 6 OF 6 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 89140732 EMBASE
DOCUMENT NUMBER: 1989140732

TITLE: Evidence in humans for **variant** allozymes of the no[redacted]ficient sparteine/debrisoquine [redacted]nooxygenase (P450IID1) in vitro.

AUTHOR: Tyndale R.F.; Inaba T.; Kalow W.
CORPORATE SOURCE: Department of Pharmacology, University of Toronto, Toronto,
Ont. M5S 1A8, Canada

SOURCE: Drug Metabolism and Disposition, (1989) 17/3 (334-340).
ISSN: 0090-9556 CODEN: DMDSAI
COUNTRY: United States
DOCUMENT TYPE: Journal
FILE SEGMENT: 022 Human Genetics
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Sparteine oxidation is part of a genetic polymorphism that affects the metabolism of many drugs and is under monogenic control. By examining the sparteine oxidation kinetics and the ratio of the dehydrogenated metabolites and through the use of the potent inhibitor, quinidine, two sites of metabolism were found for all 10 of the livers studied. The mean K(m) (N=10) for the quinidine-sensitive enzyme is 73 .+-. 46 (SD) .mu.M and the mean V(max) is 4.51 .+-. 4.16 nmol/mg microsomal protein/30 min, indicating a large interindividual variation. Because the polymorphic defect is due to at least three **variants** of a mRNA splicing error with consequent lack of enzyme formation [Gonzalez et al.: Nature **331**, 442 (1988)], the variation that we observed in K(m) is most likely due to variation of allozymes from extensive metabolizer alleles. The low affinity enzyme also demonstrates a large interindividual variation, is not competitively inhibited by quinidine, and produces a higher ratio of 5-dehydrosparteine to 2-dehydrosparteine than the high affinity enzyme. This low affinity enzyme must be part of a separate enzyme system from that controlling the sparteine/debrisoquine polymorphism because of its different characteristics and the 100% frequency with which it is found in the livers. The two dehydrosparteine metabolites are throught to be formed by the spontaneous breakdown of a primary metabolite. The different ratio of these two dehydrosparteines, which was found at low and high substrate concentrations, suggests that the reactions producing the primary metabolite are different between the quinidine-sensitive and -insensitive enzymes.

=> d l10 ibib ab 1-8

L10 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 1
ACCESSION NUMBER: 2000:675870 CAPLUS
DOCUMENT NUMBER: 134:14485
TITLE: **Catalytic** reductive dehalogenation of hexachloroethane by molecular **variants** of cytochrome **P450cam** (CYP101)
AUTHOR(S): Walsh, Mark E.; Kyritsis, Panos; Eady, Nigel A. J.; Hill, H. Allen O.; Wong, Luet-Lok
CORPORATE SOURCE: Department of Chemistry, Inorganic Chemistry Laboratory, University of Oxford, Oxford, OX1 3QR, UK
SOURCE: Eur. J. Biochem. (2000), 267(18), 5815-5820
CODEN: EJBCAI; ISSN: 0014-2956
PUBLISHER: Blackwell Science Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English
AB CYP101 (cytochrome P 450cam) catalyzes the oxidn. of camphor but has also been shown to catalyze the reductive dehalogenation of hexachloroethane and pentachloroethane. This reaction has potential applications in the biodegrdn. of these environmental contaminants. The hexachloroethane dehalogenation activity of CYP101 has been investigated by mutagenesis. The effects of active-site polarity and vol. were probed by combinations of active-site mutations. Increasing the active-site hydrophobicity by the Y96A and Y96F mutations strengthened hexachloroethane binding but decreased the rate of reaction. Increasing the polarity with the F87Y mutation drastically weakened hexachloroethane binding but did not affect the rate of reaction. The Y96H mutation had little effect at pH 7.4, but weakened hexachloroethane binding while increasing the rate of dehalogenation by up to 40% at pH 6.5, suggesting that the imidazole side-chain was partially protonated at pH 6.5 but not at pH 7.4. Substitutions by bulkier side-chains at F87, T101 and V247 weakened hexachloroethane binding but increased the dehalogenation rate. The effect of the individual mutations was additive in multiple mutants, and the most active mutant for hexachloroethane reductive dehalogenation at pH 7.4 was F87W-V247L (80 min-1 or 2.5 .times. the activity of the wild-type). The results suggested that the CYP101 active site shows good match with hexachloroethane, the Y96 side-chain plays an important role in both hexachloroethane binding and dehalogenation, and hexachloroethane binding and dehalogenation places conflicting demands on active-site polarity and compromises were necessary to achieve reasonable values for both.
REFERENCE COUNT: 34
REFERENCE(S): (1) Atkins, W; J Am Chem Soc 1989, V111, P2715 CAPLUS
(2) Atkins, W; J Biol Chem 1988, V263, P18842 CAPLUS
(3) Castro, C; Biochemistry 1985, V24, P204 CAPLUS
(5) Fetzner, S; Microbiol Rev 1994, V58, P641 CAPLUS
(6) Harford-Cross, C; Protein Eng 2000, V13, P121 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L10 ANSWER 2 OF 8 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V. DUPLICATE 2
ACCESSION NUMBER: 2000264080 EMBASE
TITLE: Temperature dependence of the formal reduction potential of putidaredoxin.

AUTHOR: Reipa V.; Holden M.J.; Mayhew M.P.; Vilker V.L.
CORPORATE SOURCE: V. Vilker, Biotechnology Division, National Institute, Standards and Technology, Gaithersburg, MD 20899, United States. vincent.vilker@nist.gov
SOURCE: Biochimica et Biophysica Acta - Bioenergetics, (20 Jul 2000) 1459/1 (1-9).
Refs: 40
ISSN: 0005-2728 CODEN: BBBEB4
PUBLISHER IDENT.: S 0005-2728(00)00108-0
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 027 Biophysics, Bioengineering and Medical Instrumentation
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Putidaredoxin (Pdx), a [2Fe-2S] redox protein of size M(r) 11 600, transfers two electrons in two separate steps from the flavin containing putidaredoxin reductase to the heme protein, cytochrome CYP101 in the **P450cam catalytic** cycle. It has recently come to light, through NMR measurements, that there can be appreciable differences in the Pdx conformational dynamics between its reduced and oxidized states. The redox reaction entropy, .DELTA.S(rc)(0')=(S(Pdx(r))(0')-S(Pdx(o))(0')), as determined from measurements of the **variation** in formal potential with temperature, E(0')(T), provides a measure of the strength of this influence on Pdx function. We designed a spectroelectrochemical cell using optically transparent tin oxide electrodes, without fixed or diffusible mediators, to measure E(0')(T) over the temperature range 0-40.degree.C. The results indicate that the redox reaction entropy for Pdx is biphasic, decreasing from -213.+-.27 J mol-1 K-1 over 0-27.degree.C, to -582.+-.150 J mol-1 K-1 over 27-40.degree.C. These redox reaction entropy changes are significantly more negative than the changes reported for most cytochromes, although our measurement over the temperature interval 0-27.degree.C is in the range reported for other iron-sulfur proteins. This suggests that Pdx (and other ferredoxins) is a less rigid system than monohemes, and that redox-linked changes in conformation, and/or conformational dynamics, impart to these proteins the ability to interact with a number of redox partners. Copyright (C) 2000 Elsevier Science B.V.

L10 ANSWER 3 OF 8 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1999364504 MEDLINE
DOCUMENT NUMBER: 99364504 PubMed ID: 10437793
TITLE: Proton nuclear magnetic resonance study of the binary complex of cytochrome **P450cam** and putidaredoxin: interaction and electron transfer rate analysis.
AUTHOR: Mouro C; Bondon A; Jung C; Hui Bon Hoa G; De Certaines J D; Spencer R G; Simonneaux G
CORPORATE SOURCE: Laboratoire de Chimie Organometallique et Biologique, UMR CNRS 6509, Universite de Rennes 1, France.
SOURCE: FEBS LETTERS, (1999 Jul 23) 455 (3) 302-6.
Journal code: EUH; 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990827
Last Updated on STN: 19990827
Entered Medline: 19990816

AB A 1H nuclear magnetic resonance study of the complex of cytochrome

P450cam-putidaredoxin has been performed. Isocyanide is bound to cytochrome **P450ca**[redacted]n order to increase the **stabil**[redacted]y of the protein both in the reduced and the oxidized state. Diprotein complex formation was detected through **variation** of the heme methyl proton resonances which have been assigned in the two redox states.

The electron transfer rate at equilibrium was determinated by magnetization transfer experiments. The observed rate of oxidation of reduced **cytochrome P450** by the oxidized putidaredoxin is 27 (+/- 7) per s.

L10 ANSWER 4 OF 8 MEDLINE DUPLICATE 4
ACCESSION NUMBER: 97163845 MEDLINE
DOCUMENT NUMBER: 97163845 PubMed ID: 9010600
TITLE: Rational approach to improving reductive catalysis by cytochrome **P450cam**.
AUTHOR: Manchester J I; Ornstein R L
CORPORATE SOURCE: Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352, USA.
SOURCE: BIOCHIMIE, (1996) 78 (8-9) 714-22. Ref: 58
Journal code: A14; 1264604. ISSN: 0300-9084.
PUB. COUNTRY: France
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970721
Last Updated on STN: 19970721
Entered Medline: 19970708

AB Although halogenated hydrocarbons are noted for low chemical reactivity, small amounts are toxic to humans. **Cytochromes P450** have been implicated in transforming these compounds to more reactive species, under anaerobic conditions, through reduction at the heme. A significant amount of effort has been directed toward turning this **catalytic** ability to our advantage by engineering P450 **variants** than can efficiently remediate these compounds in situ, before they come in contact with the human population. We have taken a 'rational' approach to this problem, in which a combination of theory and molecular modeling is applied to identify which properties of the enzyme have the greatest influence over reductive dehalogenation. Recent progress in this area is briefly reviewed. Two novel mutants, incorporating tryptophan (positions 87 and 396) and histidine (position 96, neutral and protonated) amino acid substitutions in the active site, are proposed and evaluated using molecular dynamics simulations. The upper bound on rate enhancement relative to wild-type is estimated in each mutant using electron transfer theory. The most significant rate enhancement is predicted for the His 96 mutant in the protonated state; while some His residues of certain proteins exhibit a pKa high enough to support a large protonated population, such information is not presently available for this proposed mutant.

L10 ANSWER 5 OF 8 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 96227673 MEDLINE
DOCUMENT NUMBER: 96227673 PubMed ID: 8637849
TITLE: Enzyme-catalyzed dehalogenation of pentachloroethane: why F87W-cytochrome **P450cam** is faster than wild type.
AUTHOR: Manchester J I; Ornstein R L
CORPORATE SOURCE: Environmental Molecular Sciences Laboratory, Pacific Northwest Laboratory, Richland, WA 99352, USA.
SOURCE: PROTEIN ENGINEERING, (1995 Aug) 8 (8) 801-7.
Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960719
Last Updated on STN: 19960822
Entered Medline: 19960710

AB Under anaerobic conditions, **cytochromes P450** can reductively dehalogenate heavily halogenated hydrocarbons, such as one- and two-carbon organic solvents. This **catalytic** capacity has drawn attention to the potential use of engineered forms of P450s in the remediation of contaminated deep subsurface ecosystems. Loida (1994, PhD Thesis, University of Illinois at Urbana-Champaign, IL) and S.G.Sligar (personal communication) have observed recently that an active-site **variant** of cytochrome **P450cam** (F87W) dechlorinates pentachloroethane approximately three times faster than the wild-type enzyme. Molecular dynamics simulations have revealed that the mutant enzyme binding pocket remains smaller, and that pentachloroethane assumes configurations closer to the heme-Fe in the F87W mutant twice as often as in the wild-type enzyme. This result is consistent with a collisional model of dehalogenation, which agrees with experimental observations [Li and Wackett (1993) Biochemistry, 32, 9355-9361] that solutions containing wild-type **P450cam** dehalogenate pentachloroethane 100 times faster than those containing free heme. The simulations suggest that it is unlikely that Trp87 significantly **stabilizes** the developing negative charge on the substrate during carbon-halogen bond reduction. The design of improved microbial enzymes that incorporate both steric and electronic effects continues for use in remediating halogenated contaminants in situ.

L10 ANSWER 6 OF 8 MEDLINE
ACCESSION NUMBER: 95261704 MEDLINE
DOCUMENT NUMBER: 95261704 PubMed ID: 7743131
TITLE: Structure and function of **cytochromes P450**: a comparative analysis of three crystal structures.
AUTHOR: Hasemann C A; Kurumbail R G; Boddupalli S S; Peterson J A; Deisenhofer J
CORPORATE SOURCE: Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas 75235-9050, USA.
CONTRACT NUMBER: GM43479 (NIGMS)
SOURCE: STRUCTURE, (1995 Jan 15) 3 (1) 41-62.
Journal code: B31; 9418985. ISSN: 0969-2126.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199506
ENTRY DATE: Entered STN: 19950621
Last Updated on STN: 19950621
Entered Medline: 19950612

AB BACKGROUND: **Cytochromes P450** catalyze the oxidation of a variety of hydrophobic substrates. Sequence identities between P450 families are generally low (10-30%), and consequently, the structure-function correlations among P450s are not clear. The crystal structures of P450terp and the hemoprotein domain of P450BM-3 were recently determined, and are compared here with the previously available structure of **P450cam**. RESULTS: The topology of all three enzymes is quite similar. The heme-binding core structure is well conserved, except for local differences in the I helices. The greatest **variation** is observed in the substrate-binding regions. The structural superposition of the proteins permits an improved sequence alignment of other P450s. The charge distribution in the three structures is similarly asymmetric and defines a molecular dipole. CONCLUSIONS: Based

on this comparison we believe that all P450s will be found to possess the same tertiary structure. The ability to precisely predict other P450 substrate-contact residues is limited by the extreme structural heterogeneity in the substrate-recognition regions. The central I-helix structures of P450terp and P450BM-3 suggest a role for helix-associated solvent molecules as a source of **catalytic** protons, distinct from the mechanism for **P450cam**. We suggest that the P450 molecular dipole might aid in both redox-partner docking and proton recruitment for catalysis.

L10 ANSWER 7 OF 8 MEDLINE DUPLICATE 6
ACCESSION NUMBER: 92245582 MEDLINE
DOCUMENT NUMBER: 92245582 PubMed ID: 1574808
TITLE: Mapping determinants of the substrate selectivities of P450
enzymes by site-directed mutagenesis.
AUTHOR: Johnson E F
CORPORATE SOURCE: Department of Molecular and Experimental Medicine, Scripps Research Institute, La Jolla, CA 92037.
CONTRACT NUMBER: GM31001 (NIGMS)
SOURCE: TRENDS IN PHARMACOLOGICAL SCIENCES, (1992 Mar) 13 (3) 122-6. Ref: 25
Journal code: WFT; 7906158. ISSN: 0165-6147.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920619
Last Updated on STN: 19920619
Entered Medline: 19920601

AB Point-mutation studies in cytochrome P450s by site-directed mutagenesis have identified key residues that can confer the **catalytic** properties of one **cytochrome P450** onto another. Most of these key residues cluster at sites that map to amino acids forming the substrate-binding site of **P450cam**, a distantly related enzyme. These sites are found on topological elements of **P450cam**, which by their surface location and lack of extensive secondary structure are likely to permit genetic **variation** without extensive disruption of the overall topology of the enzyme. If these topological features of **P450cam** are conserved in the mammalian enzymes, they are likely to accommodate the structural diversity seen for mammalian P450s in a manner that conserves a basic structure for P450 enzymes but that leads to the **catalytic** diversity seen for the mammalian enzymes.

L10 ANSWER 8 OF 8 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 91283446 MEDLINE
DOCUMENT NUMBER: 91283446 PubMed ID: 2059619
TITLE: A hypervariable region of P450IIC5 confers progesterone 21-hydroxylase activity to P450IIC1.
AUTHOR: Kronbach T; Kemper B; Johnson E F
CORPORATE SOURCE: Department of Molecular and Experimental Medicine, Research
Institute of Scripps Clinic, La Jolla, California 92037.
CONTRACT NUMBER: GM31001 (NIGMS)
GM35897 (NIGMS)
M01 RR00833 (NCRR)
SOURCE: BIOCHEMISTRY, (1991 Jun 25) 30 (25) 6097-102.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals

ENTRY MONTH: 199108
ENTRY DATE: Entered STN: 19910825
Last Updated on STN: 19970203
Entered Medline: 19910802

AB Cytochrome P450IIC5 is a hepatic progesterone 21-hydroxylase while the 95% identical P450IIC4 has a greater than 10-fold higher Km for progesterone 21-hydroxylation and the 74% identical P450IIC1 does not hydroxylate progesterone at detectable rates. Previous work demonstrated that the apparent Km of P450IIC4 for progesterone 21-hydroxylation can be markedly improved by replacing a valine at position 113 with an alanine which is present at this position in P450IIC5. In the present studies, a single point mutation in cytochrome P450IIC1 that changed valine at position 113 to alanine conferred progesterone 21-hydroxylase activity to this enzyme. Although the **catalytic** activity was less than that of P450IIC5, these results indicate the residue 113 plays a critical role in the determination of the substrate/product selectivity in subfamily IIC P450s.

By alignment with the sequence of **P450cam**, the segment of the polypeptide, residues 95-123, containing residue 113 corresponds to a substrate-contacting loop in the bacterial enzyme. The region containing residue 113, which is highly **variable** among family II P450s, may also be a substrate-contacting loop in the mammalian **cytochromes P450**. The exchange of this hypervariable region of cytochrome P450IIC1, residues 95-123, with that of P450IIC5 enhanced the 21-hydroxylase activity of the cells transfected with this chimera to levels similar to those of cells transfected with the plasmid encoding P450IIC5. Kinetic analysis of microsomes isolated from the transfected cells showed that the apparent Km for progesterone 21-hydroxylation of the chimera was indistinguishable from that of P450IIC5.(ABSTRACT TRUNCATED AT 250 WORDS)

L11 ANSWER 8 OF 18 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 97163845 MEDLINE
DOCUMENT NUMBER: 97163845 PubMed ID: 9010600
TITLE: Rational approach to improving reductive catalysis by cytochrome **P450cam**.
AUTHOR: Manchester J I; Ornstein R L
CORPORATE SOURCE: Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352, USA.
SOURCE: BIOCHIMIE, (1996) 78 (8-9) 714-22. Ref: 58
Journal code: A14; 1264604. ISSN: 0300-9084.
PUB. COUNTRY: France
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199707
ENTRY DATE: Entered STN: 19970721
Last Updated on STN: 19970721
Entered Medline: 19970708

AB Although halogenated hydrocarbons are noted for low chemical reactivity, small amounts are toxic to humans. **Cytochromes P450** have been implicated in transforming these compounds to more reactive species, under anaerobic conditions, through reduction at the heme. A significant amount of effort has been directed toward turning this catalytic ability to our advantage by engineering P450 **variants** than can efficiently remediate these compounds in situ, before they come in contact with the human population. We have taken a 'rational' approach to this problem, in which a combination of theory and molecular modeling is applied to identify which properties of the enzyme have the greatest influence over reductive dehalogenation. Recent progress in this area is briefly reviewed. Two novel mutants, incorporating tryptophan (positions 87 and 396) and histidine (position 96, neutral and protonated) amino acid substitutions in the active site, are proposed and evaluated using molecular dynamics simulations. The upper bound on rate enhancement relative to wild-type is estimated in each mutant using electron transfer theory. The most significant rate enhancement is predicted for the His 96 mutant in the protonated state; while some His residues of certain proteins exhibit a pKa high enough to support a large protonated population, such information is not presently available for this proposed mutant.

L15 ANSWER 1 OF 1 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 94089651 MEDLINE
DOCUMENT NUMBER: 94089651 PubMed ID: 8265573
TITLE: Structurally engineered cytochromes with unusual ligand-binding properties: expression of Saccharomyces cerevisiae Met-80-->Ala iso-1-cytochrome c.
AUTHOR: Lu Y; Casimiro D R; Bren K L; Richards J H; Gray H B
CORPORATE SOURCE: Beckman Institute, California Institute of Technology, Pasadena 91125.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1993 Dec 15) 90 (24) 11456-9. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940209
Last Updated on STN: 19940209
Entered Medline: 19940121

AB A strategy has been developed to express and purify a recombinant, nonfunctional axial-ligand mutant of iso-1-cytochrome c (Met-80-->Ala) in Saccharomyces cerevisiae in quantities necessary for extensive biophysical characterization. It involves coexpressing in the same plasmid (YEp213) the nonfunctional gene with a functional gene copy for complementation in a selective medium. The functional gene encodes a product with an engineered metal-chelating dihistidine site (His-39 and Leu-58-->His) that enables efficient separation of the two isoforms by immobilized metal-affinity chromatography. The purified Met-80-->Ala protein possesses a binding site for dioxygen and other exogenous ligands. Absorption spectra of several derivatives of this mutant show striking similarities to those of corresponding derivatives of **horseradish peroxidase**, myoglobin, and **cytochrome P450**. The use of a dual-gene vector for cytochrome c expression together with metal-affinity separation opens the way for the engineering of **variants** with dramatically altered structural and catalytic properties.

L17 ANSWER 29 OF 29 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 92029440 EMBASE
DOCUMENT NUMBER: 1992029440
TITLE: Molecular basis for a functionally unique cytochrome P450IIB1 **variant**.
AUTHOR: Kedzie K.M.; Balfour C.A.; Escobar G.Y.; Grimm S.W.; He Y.-A.; Pepperl D.J.; Regan J.W.; Stevens J.C.; Halpert J.R.
CORPORATE SOURCE: Dept of Pharmacology/Toxicol., College of Pharmacy, University of AZ,Tucson, AZ 85721, United States
SOURCE: Journal of Biological Chemistry, (1991) 266/33 (22515-22521).
ISSN: 0021-9258 CODEN: JBCHA3
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Liver microsomes from phenobarbital-treated rats of four inbred strains expressing distinct allelic **variants** of cytochrome P450IIB1 were analyzed. The Wistar Munich (WM) strain exhibited 5- to 10-fold lower androstenedione 16.beta.-hydroxylase activity (a specific P450IIB1 marker) than the Lewis, Wistar Kyoto, and Wistar Furth strains. The androstenedione 16.beta.-hydroxylase in the WM liver microsomes was refractory to inactivation by N-(2-p-nitrophenethyl)chlorofluoroacetamide, a selective P450IIB1 inactivator in the other three strains. Purified P450IIB1-WM was insensitive to the inactivator and exhibited 5-fold lower androstenedione 16.beta.-hydroxylase, testosterone 16-hydroxylase, and 7-ethoxycoumarin deethylase activities but the same benzphetamine demethylase activity and slightly higher androstenedione 16.alpha.-hydroxylase activity than a P450IIB1 purified from outbred Sprague-Dawley rats, which appears to correspond to the form in Lewis rats. The stereoselectivity of androstenedione 16-hydroxylation catalyzed by P450IIB1-WM (16.beta.-OH:16.alpha.-OH = 1.4) is thus distinct from that (16.beta.-OH:16.alpha.-OH = 12-15) of other P450IIB1 preparations described. A cDNA encoding P450IIB1-WM was cloned and sequenced, revealing a single amino acid substitution (Gly-478 .fwdarw.**Ala**) compared with the published sequence (Fujii-Kuriyama, Y., Mizukami, Y., Kawajiri, K., Sogawa, K., and Muramatsu, M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2793-2797). Heterologous expression of P450IIB1 and P450IIB1-WM confirmed the striking difference in androstenedione metabolite profiles, strongly implicating the involvement of **Ala**-478 in defining the distinctive catalytic properties of P450IIB1-WM.

L17 ANSWER 23 OF 29 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 94154531 EMBASE
DOCUMENT NUMBER: 1994154531
TITLE: Epoxidation of arachidonic acid as an active-site probe of cytochrome P-450 2B isoforms.
AUTHOR: Laethem R.M.; Halpert J.R.; Koop D.R.
CORPORATE SOURCE: Department of Pharmacology, Oregon Health Sciences University, Jackson Park Road,Portland, OR 97201-3098, United States
SOURCE: Biochimica et Biophysica Acta - Protein Structure and Molecular Enzymology, (1994) 1206/1 (42-48).
ISSN: 0167-4838 CODEN: BBAEDZ
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB In the present study we determined the regioselectivity of arachidonic acid epoxidation by several members of the cytochrome P-450 2B subfamily, including rat P-450 2B1, 2B1-WM (an allelic **variant** of 2B1 expressed in Wistar-Munich rats), 2B2, and rabbit 2B4 and 2B5. The major products formed with all isoforms were the four regioisomeric epoxides, but each isoform produced a distinct distribution of the four epoxides. P-450 2B1 produced predominantly 14,15-epoxyeicosatrienoic acid (EET), while P-450 2B1-WM produced the 11,12-EET as the major product. P-450 2B2, 2B4, and 2B5 catalyzed the formation of all four epoxides in nearly equal amounts. The single Gly-478.fwdarw.**Ala** substitution in the **variant** P-450 2B1-WM was sufficient to cause a dramatic change in the ratio of epoxides when compared with P-450 2B1. The Gly-478.fwdarw.**Ala** mutation also changed the regioselective epoxidation of .gamma.-linolenic acid at the three double bonds. Four site-directed mutants of P-450 2B1 were also evaluated. The mutations included two single mutants where Ile-114 was changed to either Val or **Ala** and two double mutants where the **Ala**-478 mutation was coupled with either Val or **Ala** at position 114. When Ile-114 was mutated to Val, the degree of epoxidation of arachidonic acid at all four double bonds was nearly equal. However, substitution of Ile-114 with **Ala**, resulted in a significant reduction in the degree of epoxidation at the 14,15- and 11,12-double bonds, and the 8,9- and 5,6-EETs were the major products. When **Ala** was introduced at position 478 in conjunction with Val at position 114 the regioselective epoxidation of the mutant enzyme more closely resembled P-450 2B1-WM in that 11,12-EET was the major metabolite. The double mutation with **Ala** at both positions 114 and 478 produced a unique distribution of epoxide products with 5,6-EET as the major metabolite. The results of these studies indicate that residues 114 and 478 in the P-450 2B subfamily are important for the orientation of fatty acids in the active site.

L17 ANSWER 11 OF 29 MEDLINE DUPLICATE 5

ACCESSION NUMBER: 1999386660 MEDLINE
DOCUMENT NUMBER: 99386660 PubMed ID: 10455016
TITLE: Lysine mutagenesis identifies cationic charges of human CYP17 that interact with cytochrome b5 to promote male sex-hormone biosynthesis.
AUTHOR: Lee-Robichaud P; Akhtar M E; Akhtar M
CORPORATE SOURCE: Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, U.K.
SOURCE: BIOCHEMICAL JOURNAL, (1999 Sep 1) 342 (Pt 2) 309-12. Journal code: 9YO; 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199911
ENTRY DATE: Entered STN: 20000111
Last Updated on STN: 20000111
Entered Medline: 19991101

AB Human CYP17 (17alpha-hydroxylase-17,20-lyase; also cytochrome P450c17 or **cytochrome P450**(17alpha)) catalyses a hydroxylation reaction and another reaction involving the cleavage of a C-C bond (the lyase activity) that is required only for androgen production. Single amino acid mutations in human CYP17, Arg(347)-->His and Arg(358)-->Gln, have been reported to result in the loss of the lyase activity and to cause sexual phenotypic changes in 46XY male patients. By using site-directed mutagenesis we show here that another mutation in human CYP17, Arg(449)-->**Ala**, for which human **variants** have yet not been described, also leads to selective lyase deficiency. Furthermore, all the three types of mutants display a loss of responsiveness to cytochrome b(5), an interaction that is essential for lyase activity, and hence male sex-hormone biosynthesis. That the defect could be essentially reversed by lysine mutagenesis has led to the conclusion that the cationic charges on all three residues (at the positions of Arg(347), Arg(358), Arg(449)) are vital for the functional interaction of CYP17 with cytochrome b(5) and that the loss of any one of these cationic charges is catastrophic.

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L21 ANSWER 1 OF 2 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 2000175739 MEDLINE
DOCUMENT NUMBER: 20175739 PubMed ID: 10708651
TITLE: Protein engineering of **cytochrome p450** (cam) (CYP101) for the oxidation of polycyclic **aromatic** hydrocarbons.
AUTHOR: Harford-Cross C F; Carmichael A B; Allan F K; England P A; Rouch D A; Wong L L
CORPORATE SOURCE: Department of Chemistry, Inorganic Chemistry Laboratory,South Parks Road, Oxford OX1 3QR, UK.
SOURCE: PROTEIN ENGINEERING, (2000 Feb) 13 (2) 121-8.
Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200005
ENTRY DATE: Entered STN: 20000525
Last Updated on STN: 20000525
Entered Medline: 20000518

AB Mutations of the active site residues F87 and Y96 greatly enhanced the activity of **cytochrome P450**(cam) (CYP101) from Pseudomonas putida for the oxidation of the polycyclic **aromatic** hydrocarbons phenanthrene, fluoranthene, pyrene and benzo[a]pyrene. Wild-type P450(cam) had low (<0.01 min(-1)) activity with these substrates. Phenanthrene was oxidized to 1-, 2-, 3- and 4-phenanthrol, while fluoranthene gave mainly 3-fluoranthol. Pyrene was oxidized to 1-pyrenol and then to 1,6- and 1,8-pyrenequinone, with small amounts of 2-pyrenol also formed with the Y96A mutant. Benzo[a]pyrene gave 3-hydroxybenzo[a]pyrene as the major product. The NADH oxidation rate of the mutants with phenanthrene was as high as 374 min(-1), which was 31% of the **camphor** oxidation rate by wild-type P450(cam), and with fluoranthene the fastest rate was 144 min(-1). The oxidation of phenanthrene and fluoranthene were highly uncoupled, with highest couplings of 1.3 and 3.1%, respectively. The highest coupling efficiency for pyrene oxidation was a reasonable 23%, but the NADH turnover rate was slow. The product distributions varied significantly between mutants, suggesting that substrate binding orientations can be manipulated by protein engineering, and that genetic **variants** of P450(cam) may be useful for studying the oxidation of polycyclic **aromatic** hydrocarbons by P450 enzymes.

L21 ANSWER 2 OF 2 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.DUPLICATE 2
ACCESSION NUMBER: 92368753 EMBASE
DOCUMENT NUMBER: 1992368753
TITLE: Genetic **variants** in the putidaredoxin-cytochrome P-450(cam) electron- transfer complex: Identification of the residue responsible for redox-state- dependent conformers.
AUTHOR: Davies M.D.; Sligar S.G.
CORPORATE SOURCE: Department of Biochemistry, University of Illinois,Urbana, IL 61801, United States
SOURCE: Biochemistry, (1992) 31/46 (11383-11389).
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States

DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 02 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB **Camphor** is hydroxylated in Pseudomonas putida by a three-component system comprised of an oxidase, cytochrome P-450(cam), and a two-protein electron- transfer chain, putidaredoxin and putidaredoxin reductase [Tyson et al. (1972) J. Biol. Chem. 274, 5777-5784]. The enzymatic removal of putidaredoxin's C-terminal tryptophan is known to cause a much reduced rate of enzymatic activity in the reconstituted **camphor** hydroxylase system [Sligar et al. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 3906-3910]. To further study the role of tryptophan in the association and/or electron-transfer reactions of putidaredoxin, the gene coding for the iron-sulfur protein was altered so that the tryptophan codon was either deleted or replaced by Phe, Tyr, Asp, Leu, Val, or Lys. Although the initial evaluation of these **variant** proteins [Davies et al. (1990) J. Am. Chem. Soc. 112, 7396-7398] showed much reduced velocities of electron transfer between P-450(cam) and the nonaromatic C- terminal proteins, the relative contributions of the binding specificity and intracomplex electron-transfer rates were not addressed. We report here a complete kinetic characterization of these proteins where the dependence of the rate constant on the putidaredoxin concentration was used to determine the intracomplex electron-transfer rate constants and the association energies for all the putidaredoxins in both oxidation states. The sum of forward and reverse intracomplex electron-transfer rate constants varies from 4.90s-1 for the Lys C-terminal **variant** to 172s-1 for the native protein. Differences in the behavior of the **variant** proteins are most striking when comparing the cytochrome P-450(cam) association energies with reduced putidaredoxins. The presence of a C-terminal **aromatic** residue is required for a relatively high cytochrome P-450(cam) affinity of the reduced relative to the oxidized protein. The desolvation of putidaredoxin's C-terminal residue is discussed as a possible explanation for this behavior.

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USPT,PGPB,JPAB,EPAB,DWPI	l10 and (identification or detection)	80	L13
USPT,PGPB,JPAB,EPAB,DWPI	l10 and (horseradish peroxidase)	85	L12
USPT,PGPB,JPAB,EPAB,DWPI	l10 and (napthalene)	1	L11
USPT,PGPB,JPAB,EPAB,DWPI	l2 and (napthalene or horseradish peroxidase or delta aminolevulenic acid)	86	L10
USPT,PGPB,JPAB,EPAB,DWPI	l6 and (glu 331 or arg 280 or cys 242)	0	L9
USPT,PGPB,JPAB,EPAB,DWPI	l6 and (glu331 or arg280 or cys242)	0	L8
USPT,PGPB,JPAB,EPAB,DWPI	l6 and (331 or 280 or 242)	158	L7
USPT,PGPB,JPAB,EPAB,DWPI	l2 and (variants or mutants)	337	L6
USPT,PGPB,JPAB,EPAB,DWPI	l2 and variants	212	L5
USPT,PGPB,JPAB,EPAB,DWPI	l3 and (331 or 280 or 242)	0	L4
USPT,PGPB,JPAB,EPAB,DWPI	l2 and P450cam	6	L3
USPT,PGPB,JPAB,EPAB,DWPI	cytochrome adj P450	987	L2
USPT,PGPB,JPAB,EPAB,DWPI	cytochrome adj P450 adj oxygenase	2	L1